

Q1 --Polycystic kidney disease (PKD) is a common inherited condition for which there are no cures and few effective therapies. The disease can be transmitted as an autosomal dominant or recessive defect. The dominant form of PKD is one of the most prevalent life-threatening genetic diseases, affecting approximately 600,000 Americans and more than 12 million families worldwide. The National Institutes of Health estimates that one in 400 to 1,000 persons has autosomal dominant polycystic kidney disease (ADPKD), and one in 10,000 to 40,000 individuals has autosomal recessive polycystic kidney disease (ARPKD). More than fifty percent of the affected individuals are expected to develop renal failure by the age of 60; consequently, ADPKD currently accounts for 4 to 8 percent of the renal dialysis and transplantation cases in the United States and Europe (Robinson and Hawkins, eds. (1981) PROC. EUROPEAN DIALYSIS AND TRANSPLANT ASSN. 17:20).--

Please replace the paragraph beginning at page 2, line 1, with the following rewritten paragraph:

Q2 -- Most forms of PKD are characterized by the development of fluid-filled cysts from the nephrons and collecting ducts of affected kidney tissue, which results in grossly enlarged kidneys with progressively weakened renal-concentration ability. Cyst development can also occur in other ductal organs such as liver, pancreas and spleen. Further systemic manifestations may include gastrointestinal, cardiovascular, and musculoskeletal abnormalities, such as colonic diverticulitis, berry aneurysms, hernias, and mitral valve prolapse (Gabow, et al. (1989) Adv. Nephrol. 18:19-32 and Gabow (1993) New Eng. J. Med. 329:332-342). Hypertension and endocrine dysfunction are also common in ADPKD patients, appearing even before symptoms of renal insufficiency.--

Please replace the paragraph beginning at page 2, line 11, with the following rewritten paragraph:

Q3 -- Recently, a few genetic attributes of PKD have been identified. Linkage studies and mutation analysis have indicated a causative gene (PKD1) located on chromosome 16p13.3, which is responsible for eighty-five percent of ADPKD cases (Reeders et al. (1985) Nature 317:542-544; Breuning et al. (1987) Lancet 2(8572):1359-1361). A large number of mutations in the PKD1 gene sequences have been found to be associated with the onset of polycystic kidney disease. Apart from large genomic deletions that eliminate PKD1, the mutations that have been defined clearly in ADPKD1 families appear to result in the transcription of a truncated or abnormal message RNA from the affected allele (The American PKD1 Consortium (1995) Human Mol. Genet. 4:575-582). These gene sequence alterations include small in-frame deletions, deletions and missense mutations that result in premature termination, splice-site mutations and chromosomal translocations which interrupt the gene. Most of the other ADPKD cases can be attributed to PKD2 (Kimberling W.J. et al. (1993) Genomics 18:467-472; Mochizuki T. et al. (1996) Science 272:1339-1342), with less than one percent due to the third locus for ADPKD, which has not been mapped yet.--

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Please replace the paragraph beginning at page 3, line 9, with the following rewritten paragraph:

24 -- Highly conserved motifs residing in the N-terminal extracellular domain include two leucine-rich repeats (LRRs) with cysteine-rich flanking regions, immunoglobulin (Ig)-like repeats, and a C-type lectin domain. Leucine-rich repeats (LRRs) are commonly found in the leucine-rich glycoprotein family, which takes part in a diversity of physiological events. Proteins sharing this homology include but are not limited to  $\alpha$ 2-glycoprotein, members of the GPIb.LX complex (von Willebrand factor receptor), *Drosophila chaoptin*, toll and slit (The American PKD1 Consortium (1995) Human Mol. Genet. 4:575-82). Many LRR proteins are localized in the plasma membrane or extracellular matrix and are thought to be involved in cell adhesion and developmental regulation (Kobe et al. (1994) Trends Biochem. Sci. 19:415-21). At least half of the LRR-containing proteins identified thus far have been shown to be involved in signal transduction, as for example the receptor tyrosine kinases Trk, TrkB, and TrkC. In addition, C-type lectin domains are known to mediate calcium-dependent, carbohydrate binding in cell-cell and cell-matrix adhesion (The International Polycystic Kidney Disease Consortium (1995) Cell 81:289-98).--

Please replace the paragraph beginning at page 4, line 2, with the following rewritten paragraph:

25 -- Elucidation of the biological functions of a gene often begins with examining the expression pattern of the gene product. Polyclonal and monoclonal antibodies directed against peptide or fusion proteins, mainly from the C-terminal region of polycystin, have been used to study the expression of polycystin in human and animal tissues (Ward et al. (1996) Proc. Natl. Acad. Sci. USA 93:1524-1528; Griffin et al. (1996) Proc. Assoc. Am. Physicians 108:185-197; Peters et al. (1996) Lab. Invest. 75:221-230; Geng et al. (1996) et. Clin. Invest. 98:2674-2682; Palsson et al. (1996) Molec. Med. 2:702-711; Van Adelsberg et al. (1997) Am. J. Physiol. 272:F602-F609; Ibraghimov-Beskrovnaya et al. (1997) Proc. Natl. Acad. Sci. USA 94:6397-6402; Geng et al. (1997) Am. J. Physiol. 272:F451-F459; Griffin et al. (1997) Kidney Int. 52:1196-1205; Geng et al. (1997) J. Am. Soc. Nephrol. 8:372A). These studies indicate that polycystin is expressed in many tissues in addition to the kidney and the liver. These include the epithelial cells of pancreatic and mammary ducts, intestinal crypts, urothelium and bronchioles; basal keratinocytes of the skin; neural crest, brain, neural plexuses and adrenal medulla; myocardium vascular smooth muscle of elastic and distributive arteries; and certain endothelial cells (Griffin et al. (1996) Proc. Assoc. Am. Physicians 108:185-197; Geng et al. (1996) J. Clin. Invest. 98:2674-2682; Ibraghimov-Beskrovnaya et al. (1997) Proc. Natl. Acad. Sci. USA 94:6397-6402; Geng et al. (1997) Am. J. Physiol. 272:F451-F459; Griffin et al. (1997) Kidney Int. 52:1196-1205; Griffin et al. (1997) J. Am. Soc. Nephrol. 8:616-626; O'Sullivan et al. (1997) J. Am. Soc. Nephrol.

8:376A). Studies on the immunolocalization of polycystin in the kidney, however, yielded ambiguous results. For instance, there are conflicting observations as to whether polycystin is expressed in the glomeruli region of the kidney nephrons. There are also differing views as to whether polycystin is localized to basal and apical membranes of renal epithelial cells, and to what degree it is present in the cytoplasm.--

Please replace the paragraph beginning at page 43, line 22, with the following rewritten paragraph:

-- The polycystic kidney disease 1 (PKD1) gene encodes a novel protein with multiple cell recognition domains. Hughes J. et al. (1995) Cell 10:151-160. The analysis of the three-dimensional structure of a single repeat showed that it is not a true member of Ig superfamily, although it has a characteristic  $\beta$ -sandwich topology. Bycroft M. et al. (1999) EMBO J. 18:297-305. Domains with this Ig-like fold are present in proteins as diverse as matrix proteins, receptors and enzymes, and in each case they have been shown to interact with extremely different ligands varying from small peptides (e.g., HLA) to giant proteins (e.g., titin oligomer). Bork A. et al. (1994) J. Mol. Biol. 242 :309-320.--

Please replace the paragraph beginning at page 44, line 1, with the following rewritten paragraph:

-- Using antibodies against three different regions of polycystin-1: N-terminal (LRR), C-terminal, and the middle region (REJ), the experiments described herein clearly showed that polycystin-1 was predominantly expressed at sites of cell-cell contact in kidney epithelial cells, as was the case for endothelial cells. The homophilic binding potential of several Ig-like domains, i.e., Ig<sup>a</sup>, Ig<sup>b</sup> and Ig<sup>c</sup>, containing 4, 5 and 6 domains, as clusters were analyzed as described below. Each region was translated *in vitro* and tested for the ability to bind to each region including itself in the form of immobilized fusion protein. The binding properties of all combinations were quantitatively analyzed as a percentage of binding of *in vitro* translated protein. In this type of assay the fusion proteins are present in a vast excess compared to the amount of the translated probe. Therefore, theoretically almost all of the translated probe should bind to immobilized fusion protein, even if binding is weak. Phizicky, E.M. & Fields, S. (1995) Microbiological Reviews 59:94-123. In practice, deviations from quantitative binding occur if not all of the immobilized protein or/and *in vitro* translated probe is functionally active. Nevertheless, a functionally relevant interaction should result in significant retention of ligand. For example, estimates from affinity chromatography binding experiments on the N-NusA, NusA-RNA polymerase and RAP30/74-RNA polymerase II interactions indicate that at least 50% of these proteins are available for binding. Formosa, T. et al. (1991) Meth. Enzymol. 208:24-45.--

Please replace the paragraph beginning at page 45, line 15, with the following rewritten paragraph:

Q2 -- To adequately assess the significance of the Ig-like domain homophilic interactions under consideration, they were compared them side by side with known interactions. One of those was the interaction between p53 and SV40 large T-antigen, which is known to be functionally significant. Lane D.P. et al. (1979) Nature 278:261-263 and Iwabuchi K. et al. (1993) Oncogene 8:1693-1696. The bound fraction of T-antigen comprised approximately 45% of the total probe in this system. The interaction between the PKD1 and PKD2 gene products also was used as a reference. Qian F. et al. (1997) Nature Genetics 16:179-183 and Tsiokas L. et al. (1997) Proc. Natl. Acad. Sci. USA 94:6965-6970. This interaction was initially identified by the two-hybrid assay and was further characterized using the *in vitro* binding assay. Approximately 1.5% of the input polycystin-1 probe bound to immobilized polycystin-2, while 6% of the labeled ligand was bound in the reverse combination. Qian F. et al. (1997) Nature Genetics 16:179-183. Similarly, a weak PKD2-PKD1 gene product interaction was detected which never exceeded ~1% of binding in different buffer compositions. Thus, the strength of the homophilic interactions between the various Ig-like regions of polycystin-1 as measured *in vitro* is more comparable to the known functionally significant p53-T antigen binding rather than to the weaker and likely transient interaction between polycystin- 1 and -2.--

Please replace the paragraph beginning at page 46, line 11, with the following rewritten paragraph:

Q9 --The formation and progression of ADPKD cysts is characterized by increased cell proliferation, resulting in expansion of the epithelium, which displays a relatively undifferentiated appearance. Grantham J. (1996) Amer. J. Kidney Diseases 28:788-803 and Avner E.D. (1993) J. Cell Sci. 17:217-222. The role of polycystin-1 in mediating cell-cell interactions, where such interactions are fundamental for cellular functions of proliferation, differentiation and maturation, is supported by a recent study of a targeted PKD1 mutation in mice. Lu W. et al. (1997) Nature Genetics 17:179-181. This study demonstrates that polycystin-1 is critical in the establishment and maturation of normal tubular architecture. Lu W. et al. (1997), *supra*. It has been shown that the expression of polycystin-1 is continued into adult life at a lower level, where its functional activity might be required for cells to remain tightly associated in the epithelium. Peters D.J.M. et al. (1996) Laboratory Investigation 75:221-230; Ibraghimov-Beskrovnaya O. et al. (1997) Proc. Natl. Acad. Sci. USA 94:6397-6402; Weston B.S. et al. (1997) Histochemical Journal 29:847-856; and Ward C.J. et al. (1996) Proc. Natl. Acad. Sci. USA 93:1524-1528. In addition, it is known that cell adhesion proteins play an important role in intercellular signaling. Gumbiner B.M. (1996) Cell 84:345-3 57. The results presented herein show that the loss of intercellular interactions due to a mutated polycystin-1 can be an important step in molecular cystogenesis.-